

AMENDMENTS TO CLAIMS

Please amend claims 1, 11, 48 and 56, and cancel claims 10 and 22-23 without prejudice, as below:

1. (currently amended) A method of altering gene expression in a population of human embryonic stem cells; comprising:
introducing a transfection preparation comprising a polynucleotide into the population of cells,
wherein (a) the polynucleotide is operably linked to a promoter and ~~containing~~ contains a gene expression altering sequence so that gene expression in the embryonic stem cells prior to introducing the polynucleotide is measurably different from gene expression after introducing the polynucleotide while retaining the pluripotent character of the cells; and (b) the transfection preparation further comprises one or more transfection reagents selected from the group consisting of a cationic non-lipid polymer reagent, a non-liposomal reagent, a cationic lipid agent.
2. (previously presented) The method according to claim 1, wherein the expression altering sequence is an enhancer sequence for modulating gene expression in the population of embryonic stem cells.
3. (previously presented) The method according to claim 1, wherein the expression altering sequence is a gene encoding a protein, the protein not expressed in the population of embryonic stem cells absent the polynucleotide.
4. (previously presented) The method according to claim 3, wherein the protein is selected from a fluorescent protein and an antibiotic resistance protein.
5. (previously presented) The method according to claim 4, wherein the

fluorescent protein is selected from green fluorescent protein, lacZ, firefly Rennilla protein, luciferase, red cyan protein and yellow cyan protein.

6. (previously presented) The method according to claim 4, wherein the antibiotic resistance protein is selected from hygromycin, neomycin, zeocin and puromycin.
7. (previously presented) The method according to claim 1, wherein the polynucleotide is formulated with a cationic non-lipid polymer transfection reagent for introducing the polynucleotide into the population of cells.
8. (previously presented) The method according to claim 1, wherein the polynucleotide is formulated with a non-liposomal transfection reagent for introducing the polynucleotide into the population of cells.
9. (previously presented) The method according to claim 1, wherein the polynucleotide is formulated with a cationic lipid reagent for introducing the polynucleotide into the population of cells.
10. (Cancelled)
11. (currently amended) A method of altering gene expression in a population of human embryonic stem cells; comprising:
 - introducing into the population of cells ~~in the presence of a cationic polymer,~~ a transfection preparation comprising a DNA sequence operably linked to a promoter and corresponding to at least one of an enhancer and a gene so as to alter gene expression in the population of embryonic cells in an amount to permit cells containing the DNA sequence to be distinguished from cells absent the DNA sequence,
 - wherein the transfection preparation further comprises one or more transfection reagents selected from the group consisting of cationic polymer agents.

12. (original) A method according to claim 11, wherein the DNA sequence corresponds to a gene and the gene encodes a protein selected from a fluorescent protein, a suicide gene, and an antibiotic resistance protein.
13. (previously presented) A method according to claim 11, wherein the promoter is selected from rex-1, oct-4, oct-6, SSEA-3, SSEA-4, TRA1-60, TR1-81, GCTM-2, alkaline phosphatase, and Hes1 promoters.
14. (original) A method according to claim 12, wherein the fluorescent protein is selected from green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan protein and yellow cyan protein.
15. (original) A method according to claim 12, wherein the protein is an antibiotic resistance protein and the antibiotic resistance protein is selected from hygromycin, neomycin, zeocin and puromycin.
16. (original) A method according to claim 12, wherein the DNA corresponds to a suicide gene and the suicide gene is an inducible apoptic gene or encodes a protein selected from herpes simplex thymidine kinase, inducible Diptheria toxin, bacterial cytosine deaminase.
17. (previously presented) A method according to claim 11, wherein the DNA sequence causes a knockout of a genomic sequence, the genomic sequence selected from beta 2 microglobulin, HLA-1, HLA-2 or an INF receptor gene sequence.
18. (withdrawn) A method for purifying pluripotent embryonic stem cells from a heterogeneous population of cells, comprising:
 - (a) introducing into the cells, a DNA encoding a selectable marker under a

- promoter that is specifically active in undifferentiated cell;
 - (b) separating those cells expressing the selectable marker from cells not expressing the marker; and
 - (c) obtaining purified pluripotent cells.
19. (withdrawn) A method according to claim 18, wherein the selectable marker is a fluorescent marker.
20. (withdrawn) A method according to claim 18, wherein the fluorescent marker is selected from green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan protein and yellow cyan protein.
21. (withdrawn) A method according to claim 18, wherein (b) further comprises separating the cells containing the marker from the cells lacking the marker using a fluorescent activated cell sorter or a laser scanning cytometer.
- 22-23. (Cancelled)
24. (withdrawn) A method for treating a human subject for a condition resulting from a deficiency of a selected cell type, comprising:
- (a) causing human embryonic stem cells to be transfected *in vitro* with a nucleic acid encoding a marker under a tissue specific promoter;
 - (b) separating the selected cell type expressing the marker from cells not expressing the marker, and
 - (c) introducing the selected cell type into the subject so as to treat the condition.
25. (withdrawn) A method according to claim 24, wherein the nucleic acid further contains a suicide gene.

26. (withdrawn) A method according to claim 25, wherein the suicide gene is an inducible apoptic gene or encodes a protein selected from herpes simplex thymidine kinase, inducible Diphtheria toxin, and bacterial cytosine deaminase.

27. (withdrawn) A method according to claim 24, wherein the marker is selected from a fluorescent marker and an antibiotic resistance protein.

28. (withdrawn) A method according to claim 27, wherein the fluorescent protein is selected from green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan protein and yellow cyan protein.

29. (withdrawn) A method according to claim 27, wherein the antibiotic resistance protein is selected from hygromycin, neomycin, zeocin and puromycin.

30. (withdrawn) A method according to claim 24, wherein the cells are transfected by means of a cationic polymer transfection reagent.

31. (withdrawn) A method according to claim 24, wherein the nucleic acid does not contain viral genes.

32. (withdrawn) A method according to claim 24, wherein the cell type is selected from epidermal cells, dermal cells, muscle cells, cartilage cells, osteoblasts, osteoclasts, neurons, retinal cells, endodermal cells, hematopoietic cells, cells of the immune system.

33. (withdrawn) A method according to claim 24, wherein the cell type is selected from specialized cells from any functionally distinct organ in the human subject.

34. (withdrawn) A method according to claim 24, wherein introducing the cell type to the subject is achieved by injection.

35. (withdrawn) A method according to claim 24, wherein the condition is selected from cancer, an immune disorder, an autoimmune disease, a disease of aging, a degenerative disease including a neurodegenerative disease, and trauma.

36. (currently amended) A cell population[[:]]having altered gene expression produced in accordance with claim 1 comprising a substantially pure population of human embryonic stem cells containing an expression altering sequence of exogenous DNA.

37. (withdrawn) A method of producing a clonal pluripotent cell population from a mixture of pluripotent and differentiated cells; comprising:

- (d) transfecting the mixture of cells in the presence of a cationic polymer or by electroporation with a DNA encoding a marker protein under a promoter that is selectively active in cells of the inner cell mass of the embryo; and
- (e) separating the embryonic stem cells from the differentiated cells according to the presence or absence of an expressed marker so as to produce the clonal pluripotent cell population.

38. (withdrawn) A method according to claim 37, wherein the promoter selected from rex-1, oct-4, oct-6, SSEA-3, SSEA-4, TRA1-60, TR1-81, GCTM-2, alkaline phosphatase, and Hes1 promoters.

39. (withdrawn) A method according to claim 37, wherein the cell population is transfected with a DNA in the presence of a cationic polymer.

40. (withdrawn) A method according to claim 37, wherein the marker protein is selected from a fluorescent protein and an antibiotic resistance protein.

41. (withdrawn) A method according to claim 37, wherein the fluorescent protein is selected from green fluorescent protein, lacZ, firefly Rennila protein,

luciferase, red cyan protein and yellow cyan protein.

42. (withdrawn) A method according to claim 37, wherein the antibiotic resistance protein is selected from hygromycin, neomycin, zeocin and puromycin.

43. (withdrawn) A method of regulating cell viability of a population of cells in a subject, wherein the population of cells are derived from a human embryonic stem cell culture which has undergone directed differentiation, comprising:

- (f) introducing the population of cells into the subject, the population of cells containing an exogenous DNA encoding a suicide gene, wherein the population of cells are selected from the group consisting of: undifferentiated cells, partially differentiated or wholly differentiated cells; and
- (g) treating the subject with an agent for activating a sequence of events leading to suicide in the cells in the subject in response to an adverse event associated with the introduced cells.

44. (withdrawn) A method according to claim 43, wherein the occurrence of adverse events is a hyperproliferation of the introduced cells.

45. (withdrawn) A method for screening an agent to determine an effect on differentiation of pluripotent cells *in vitro*, comprising:

- (a) adding the agent to an *in vitro* cell culture of a population of genetically engineered human embryonic stem cells expressing a detectable marker under a cell specific promoter; and
- (b) providing the conditions for the embryonic stem cells to differentiate; and
- (c) determining the effect of the agent on differentiation of pluripotent cells.

46. (withdrawn) A method according to claim 45, wherein the detectable marker is a fluorescent marker.

47. (withdrawn) A method according to claim 46, wherein the fluorescent marker is enhanced green fluorescent protein.
48. (currently amended) A reagent cell population for supplying material for transplantation having altered gene expression and produced in accordance with claim 1 consisting essentially of pluripotent human embryonic stem cells modified by ~~foreign~~ genetic material which is DNA not normally present in embryonic stem cells; which occurs in embryonic stem cells but is not expressed in them at levels which are biologically significant; DNA which occurs in embryonic stem cells and has been modified so that it is only expressed by selected cells derived from transfected human embryonic stem cells; or any DNA that can be modified to be expressed by embryonic cells, derivative cells alone or in any combination thereof.
49. (original) A reagent cell population according to claim 48, in which the foreign genetic material comprises genetic material encoding at least one selectable marker.
50. (original) A reagent cell population according to claim 49, in which at least one selectable marker is a dominant selectable marker.
51. (original) A reagent cell population according to claim 50, in which the dominant selectable marker is a gene encoding antibiotic resistance.
52. (original) A reagent cell population according to claim 49, in which the dominant selectable marker is a gene encoding a suicide protein.
53. (original) A reagent cell population according to claim 50, in which the dominant selectable marker is a gene encoding a fluorescent protein or an antibiotic resistant protein.

54. (original) A reagent cell population according to claim 52, in which the gene is *hsv-tk* and the suicide protein is thymidine kinase or the suicide gene is an inducible apoptic gene or encodes a protein selected from inducible Diphtheria toxin, bacterial cytosine deaminase

55. (original) A reagent cell population according to claim 53, wherein the gene encodes a fluorescent protein selected from green fluorescent protein, lacZ, firefly Rennila protein, luciferase, red cyan protein and yellow cyan protein.

56. (currently amended) A ~~method~~ reagent cell population according to claim 53, wherein the antibiotic resistance protein is selected from hygromycin, neomycin, zeocin and puromycin.

Claims 57-58. Not Entered.